diseases and certain prion diseases. In addition, autophagy is unique as a mechanism that can remove entire organelles, an important task beyond the abilities of the proteasome that allows for the removal of damaged or obsolete organelles, potentially eliminating oxidative stress or allowing cellular remodeling.

Do we know anything about its regulation? A little. In animal cells, components of the class I phosphatidylinositol (PI) 3-kinase pathway including Akt and Tor act as inhibitors, whereas the lipid phosphatase PTEN, class III PI 3-kinase and p70 S6 kinase appear to be positive regulators. In yeast, Tor kinase and protein kinase A are inhibitory, while PI 3-kinase is required for autophagy. Many questions remain regarding the networked interactions that control autophagic responses to different stimuli.

What else do we know? Many autophagy-specific proteins have been identified in yeast and shown to have orthologs in higher eukaryotes, including two systems that involve ubiquitin-like proteins. One of them, Atg8, modifies phosphatidylethanolamine and may act as a structural component of the autophagosome. A further 27 other proteins that act only in autophagy have been identified. In yeast, most of these proteins localize at least transiently to the pre-autophagosomal structure, which may be the site of autophagosome formation (Figure 1).

What don't we know about it? Plenty... We do not definitively know the source of the sequestering membrane (but the ER is probably involved) and, although many protein components have been identified, we don't really know the function of most of them. Because of these two limitations, we do not know how the sequestering vesicle —the hallmark of macroautophagy — is formed. Another major issue is how specificity is achieved, either for the removal of a particular organelle or the recognition of invading pathogens.

Where can I find out more?

Kirkegaard, K., Taylor, M.P. and Jackson, W.T. (2004). Cellular autophagy: surrender, avoidance and subversion by microorganisms. Nat. Rev. Microbiol. *2*, 301–314.

Levine, B., and Klionsky, D.J. (2004). Development by self-digestion: molecular mechanisms and biological functions of autophagy. Dev. Cell *6*, 463–477.

Shintani, T., and Klionsky, D.J. (2004). Autophagy in health and disease: a double-edged sword. Science *306*, 990–995.

Life Sciences Institute, University of Michigan, Ann Arbor, Michigan 48109-2216, USA. E-mail: klionsky@umich.edu

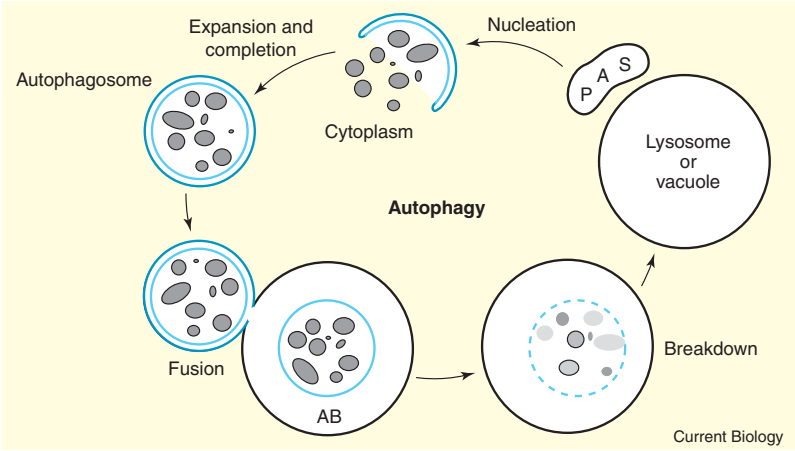

Figure 1. The process of macroautophagy.

A double-membrane-bound autophagosome sequesters cytoplasm. Fusion with the lysosome or vacuole releases the single-membrane autophagic body (AB) that is broken down, allowing degradation of the cargo and recycling of the resulting macromolecules. In yeast, most of the Atg proteins localize to the pre-autophagosomal structure (PAS), which may be the site of nucleation for the sequestering vesicle.

Primer

Selection and the origin of species

Arianne Y.K. Albert and Dolph Schluter

Why are there so many species on earth? Answering this question requires an understanding of how species form. An obvious place to start looking for answers is Darwin's 'On the Origin of Species by Means of Natural Selection' (1859). But his title is deceptive: Darwin's book is about adaptation and the origin of varieties and has surprisingly little to say about selection and "the origin of species — that mystery of mysteries".

To be fair to Darwin, it was not for another 80 years or so that the modern view of the *species* was developed. The 'biological species concept' defines a species as one or more populations of potentially interbreeding organisms that are reproductively isolated from other such groups. Humans and chimps are today separate species not only because we are genetically and phenotypically distinct, but because we are reproductively isolated. Neither finds the other attractive when choosing a mate ('premating isolation') and very likely, hybrids are inviable or sterile ('postmating isolation'). Reproductive isolation is therefore the most salient evolved feature of a species, at least in sexual organisms. Even 'good' species may hybridize once in a while, but to meet the species criterion the flow of genes between them must be negligible. The study of *speciation* is therefore the study of how reproductive isolation evolves, premating or postmating, between populations.

Natural selection is the differential survival or reproductive success of individuals differing in phenotype within a population. Sexual selection, by contrast, is the differential mating success of phenotypically different

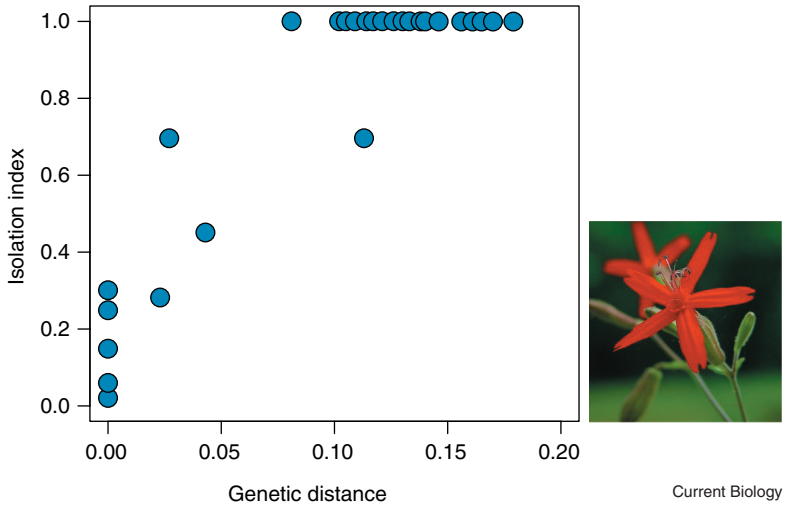

Figure 1. Reproductive isolation between species of *Silene* (campion) in relation to gene sequence differences in internal transcribed spacer (ITS) regions of the genome. The isolation index ranges from 0 (no isolation) to 1 (complete isolation). Reproductive isolation was calculated as a combination of two measures: the proportion of failed pollinations between the species and the proportion of infertile pollen grains in their F1 hybrids. Redrawn with permission from: Moyle, L.C., Olson, M.S., and Tiffin, P. (2004). Patterns of reproductive isolation in three angiosperm genera. Evolution *58*, 1195–1208. The flower image on the right is of fire pink (*Silene virginica*), native to the eastern US (photo: Dan Tenaglia).

individuals. These two processes are the most potent drivers of evolutionary change within populations. Here we shall consider some of their contributions to the buildup of reproductive incompatibilities between populations — the origin of species.

Obstacles to speciation

Speciation is intimately tied to genetic divergence between populations (Figure 1). On average, the greater the genetic distance between any pair of populations or species, the lower the frequency of mating and fertilization events, and the lower the mating success, fertility and viability of hybrids. What could be simpler to understand?

Yet, speciation is not always easy. The two main obstacles are gene flow and, paradoxically, natural selection. To appreciate both, imagine a few small-beaked finches blown to a little remote island containing both small and large seeds in mixed patches. Small beaks are best adapted to feeding on the small seeds, but a large beak increases ability to handle the large seeds. Intermediate beaks are inferior on both seed types. Imagine further that beak size is determined by two genes that behave additively: the small-beaked colonists are *aabb* whereas the optimal beak for handling large seeds is *AABB*.

This scenario appears to be ripe for a speciation event, one yielding two reproductively isolated species each adapted to a different seed size. Will it happen? Possibly yes, but probably not, for two reasons. First, evolving a large beak faces the problem that every new mutant individual — *aaBb* or *aAbb* — has lower fitness and is selected against (the fitness valley problem). Second, even if the fitness valley could somehow be crossed, and *AABB* genotypes generated, gene flow hinders the evolution of reproductive isolation between the beak types. Imagine a mating locus with two alleles: allele *p* predisposes its bearer to mate with small beaked individuals, whereas birds with allele *P* prefer large beaked individuals as mates. Natural selection will increase the frequency of *aabbpp* and *AABBPP* genotypes because they are best adapted to the environment and make the fewest inferior (heterozygote) offspring. Yet, unless reproductive isolation is largely complete in one fell swoop, sexual reproduction breaks up these favored combinations every generation (selection–recombination antagonism).

Both difficulties are easily solved by spatial separation between populations, reducing gene flow (the 'allopatric' and 'parapatric' models). Send some of the small-beaked birds to a second island having only large seeds and see a large beak evolve there without impediment. Now, an intermediate beak size improves fitness — the fitness valley is eliminated. Genetic changes that yield reproductive isolation between the populations on the different islands evolve more readily because gene flow between islands is too low to impede its build-up. With the accumulation of genetic differences between islands, sufficient reproductive isolation may evolve that the large-beaked population can recolonize and persist on the first island: two coexisting species at last.

These obstacles explain why most speciation events in nature appear to have included a stage in which populations were spatially separated. Yet, speciation *can* happen in the single-island case, without spatial separation (the 'sympatric' model). Convenient genetics can help. For example, if the alleles at a mating locus are *p* (mate randomly) and *P* (mate with another having the same beak size), then the selection–recombination antagonism goes away. Only a handful of compelling cases of sympatric speciation have been described.

The host-races of the apple maggot fly (*Rhagoletis pomonella*) provide the best known example of sympatric speciation in progress. Two host-races, one living on apples and one living on hawthorn, occur in sympatry in the northeastern United States. Hawthorn *Rhagoletis* colonized apple trees fewer than 150 years ago. The new race has already acquired adaptations to its new host, and individuals that return to the ancestral host fare badly. Females of each host-race prefer

to lay eggs on their own type of fruit, and as mating too occurs on the fruit, premating isolation has followed. It is likely that the new host-race arose and accumulated genetic differences in sympatry, but some genes underlying adaptation to apples reside in a chromosomal inversion that originated in Mexico. Northward spread of this inversion, and its associated genes, probably facilitated adaptation to apple. Selection on a mix of genetic variation produced in sympatry and allopatry appears to be driving speciation between hawthorn and apple maggot flies.

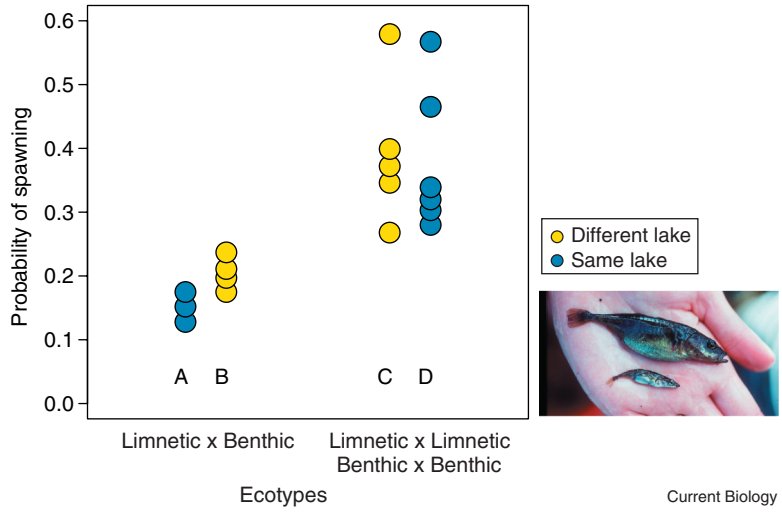

Figure 2. Probability of spawning according to ecological differences between independently evolved populations and species of threespine sticklebacks.

Points on left are from mating trials between sympatric (A) and allopatric (B) limnetics and benthics. Points on right are from trials between conspecific limnetics or conspecific benthics (D), and between allopatric limnetics or allopatric benthics (C). Spawning probability is low when different ecotypes are tested, but higher when trials involve the same ecotypes even when the populations are from different lakes. The probability of spawning is adjusted to take into account the different propensity of males from different populations to spawn in captivity. Redrawn with permission from: Rundle, H.D., Nagel, L., Boughman, J.W., and Schluter, D. (2000). Natural selection and parallel speciation in sympatric sticklebacks. Science *287*, 306-308. The larger fish in the photograph is a benthic female and the smaller fish is a limnetic female, both from Paxton Lake on Texada Island, British Columbia (photo: Todd Hatfield).

Natural selection

Natural selection is probably involved in most cases of speciation. Many scenarios are possible, but here we focus on two of the most likely. In the first, reproductive isolation evolves between populations as a by-product of *divergent* natural selection that favors different genotypes in contrasting environments. Selection does not directly favor the evolution of reproductive isolation, at least not initially. Rather, selection favors alternative morphological, physiological and behavioral traits in contrasting environments, and some of these differences incidentally yield premating and/or postmating reproductive incompatibilities between the populations. The key feature of this process is that speciation is environment driven.

Important evidence for this process comes from cases in which reproductive isolation has evolved independently multiple times across similar environmental gradients ('parallel speciation'). Threespine sticklebacks (*Gasterosteus sp.*) provide several examples. Reproductively isolated pairs of species inhabit small lakes of coastal British Columbia, Canada. Each pair consists of a large-bodied 'benthic' species adapted to feeding in the littoral habitat of lakes, and a small-bodied 'limnetic' species feeding in open water on zooplankton. Each pair appears to have an independent origin, yet reproductive isolation is strong not only between the species within a lake, but also between the phenotypically different forms from different lakes (Figure 2). In contrast, there is little reproductive isolation between populations of the same ecological type from different lakes. Reproductive isolation is therefore associated with adaptation to different environments, strongly implicating divergent natural selection in the origin of these species. The buildup of these mating incompatibilities is strongly connected to divergence in body size.

In the second scenario, different advantageous but incompatible mutations arise and fix in separate populations that otherwise inhabit similar environments (*uniform* natural selection). For example, one population might fix a mutation that improves a signaling protein and another fixes a mutation improving its binding site. A hybrid between the two populations would carry both new mutations, but its fitness might be reduced if the new protein does not bind to the new site. Reproductive isolation builds as a by-product of genetic divergence, but here not even divergence is favored by natural selection. Selection drives new mutations to fixation, but speciation happens only because of the occurrence of unique mutations in different populations.

A possible example comes from the fruit flies, *Drosophila melanogaster* and *D. simulans*. Hybrid males between the two species are inviable, a feature that has been traced to the gene *Nucleoporin-96* (*Nup96*), which encodes a protein in the nuclear pore complex. *Nup96* from *D. simulans* interacts negatively with a gene or genes on the X chromosome of *D. melanogaster*, causing death of male individuals carrying both. *Nup96* has undergone adaptive evolution in one or both species, as implied by an excess of amino acid substitutions compared with a neutral model of evolution. The question now is: what kind of selection led to the changes in *Nup96* and the X-linked gene(s)? Did different advantageous mutations really fix in the two

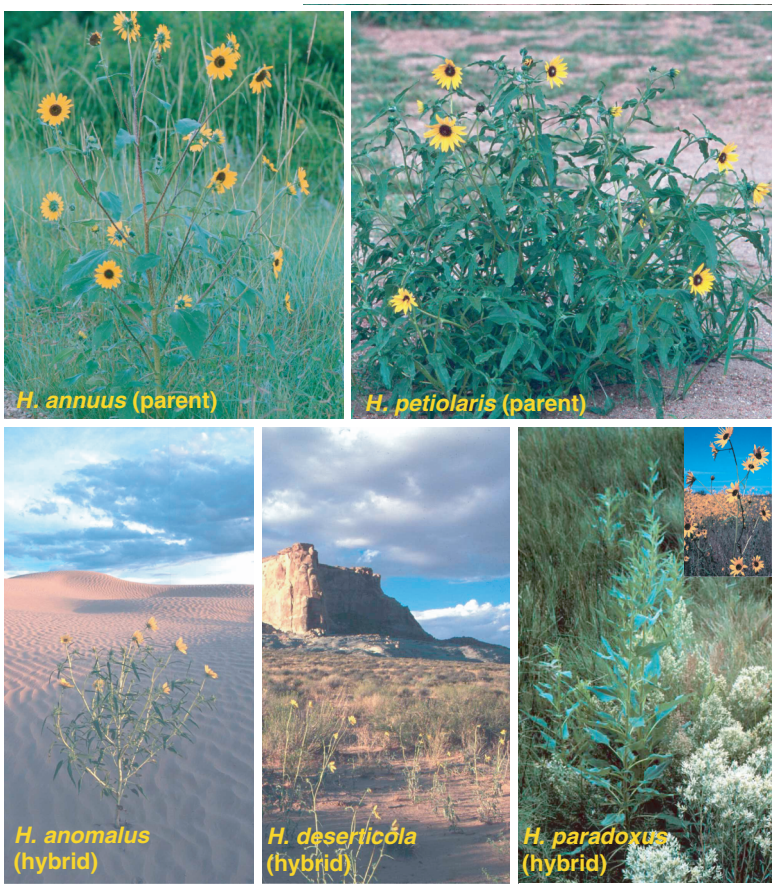

Figure 3. Three different diploid hybrid species of sunflower, each initiated by a hybridization event between *Helianthus annuus* and *H. petiolaris*.

Each hybrid species thrives in a distinct habitat that is stressful to both of the parental species. *H. paradoxus* inhabits salt marshes, *H. anomalus* inhabits sand dunes, and *H. deserticola* inhabits desert areas. (Photos: Loren Rieseberg and Jason Rick.)

Drosophila species despite similar environmental pressures, or might divergent selection have played a role? Further research will hopefully be able to answer this question.

Notably, in neither of the above scenarios does natural selection actually favor speciation. Rather, reproductive isolation evolves incidentally from the action of natural selection on ordinary traits. But everything changes when contact between the populations, now incipient species, becomes extensive after a period of divergence. If reproductive isolation is incomplete, then hybrids will be formed that have reduced survival or reproductive success. At this point the divergence built up by many generations of selection might simply collapse in the face of gene flow. But if gene flow is not too great then natural selection may be effective in strengthening premating isolation further, favoring parents that avoid matings that produce inferior hybrid offspring. This process, known as *reinforcement*, is thought to be important for finishing the process of speciation.

Reinforcement leads to the pattern whereby members of two species have stronger premating isolation in areas where they are in contact than individuals from allopatric populations of similar age. One example comes from two *Drosophila* species that co-occur in North America. *D. pseudoobscura* is widely distributed but *D. persimilis* occurs only along the Pacific coast, completely within the range of *D. pseudoobscura*. Male hybrids between these two species are sterile. As predicted by reinforcement, female *D. pseudoobscura* from the zone of overlap discriminate against *D. persimilis* males when choosing mates, whereas females outside the overlap zone discriminate less. Similar patterns have been found in other taxa, although the pattern is not universal. In most cases we still do not know how large is the effect of reinforcement in speciation relative to other processes.

Sexual selection

Sexual selection occurs when individuals of one sex (for example females) preferentially mate with members of the other sex (males) according to trait differences, or when differences between males affect competition among them for access to fertilization. The evolutionary outcome of such selection has produced the extravagant colors, sounds, genital shapes and behavioral displays that characterize male courtship and mating behavior in many animal species. The targets of selection may have nothing to do with adaptation to environment — indeed, they are usually hazardous for the male to bear.

The reason we think sexual selection is important to speciation is that so many closely related species in nature differ in these secondary sexual characters, often more so than in other traits. A classic example is the Haplochromine cichlids of Lake Victoria. Although they have diversified into many ecologically different groups, many closely related species are similar in ecology yet very different in male coloration. There is evidence that females use color to identify males of their own species and that they prefer the most colorful males. Taken together, these patterns imply that sexual selection has somehow contributed to the divergence in color, with the result that males of each species are unattractive to females of the other species.

The other evidence for sexual selection in speciation is even more indirect. It is based on the finding that animal taxa with apparently high levels of sexual selection are often excessively species rich. For example, insect

taxa in which females mate with multiple males have more species than similar-aged taxa in which females mate only once. Multiple mating provides a larger potential for sexual selection as it allows for sperm competition between males and larger variance between males in reproductive success.

For sexual selection to drive speciation, a mechanism is needed for preferences and secondary sexual traits to diverge between populations. Many mechanisms are possible, but the two most likely will sound familiar: divergent natural selection and the fixation of different advantageous mutations under similar sexual selection pressures.

Divergent natural selection can drive changes in mate preferences between two populations if they inhabit environments with contrasting signal transmission properties. For example, light quality and characteristics differ between different *Anolis* lizard habitats in the Caribbean islands, making some color signals easier to see and others more difficult. Experiments have demonstrated that female *Anolis* lizards prefer to mate with males whose throat colors transmit best in each light environment. Such divergence in preferences and signals, driven by differences in light environments, might incidentally lead to the buildup of premating isolation between populations.

Sexual conflict between males and females produces the setting for the second scenario. For example, adaptations that enhance the competitive success of a male's sperm may cause harm to the female and a reduction in her fitness. Counter-adaptations in females in turn favor males with unique sperm proteins that again have a competitive advantage. This coevolutionary sequence can eventually lead to reproductive isolation between closely related populations if sperm from one population incidentally becomes ineffective at fertilizing female eggs from the other population. Sexual selection favors change but does not directly favor divergence. Rather, divergence occurs as an inevitable side effect of the fixation of different advantageous mutations in males and females in separated populations.

Evidence of speciation by sexual conflict comes mainly from insects. For example, the eggs of female flour beetles (*Tribolium castaneum*) are preferentially fertilized by the sperm of males from their own population. This strongly suggests coevolution between male and female reproductive function, possibly because of sexual conflict. Examples from traits other than sperm–egg compatibility are few.

One step speciation

Plant speciation involves all of the above but plants also routinely speciate in a single step by a process much rarer in animals: polyploidization, or chromosome doubling. Production of unreduced gametes in hybrids between two species is often the impetus, yielding a new polyploid species combining both parental genomes but largely reproductively isolated from them by the low fertility of triploid offspring. The result is a 'hopeful monster,' a new species represented by a single individual. Its initial success is aided by the ability to self-fertilize or by the generation of other individuals by repeated independent polyploidization events. A polyploid species may similarly derive from fusion of unreduced gametes from a single parent species, without hybridization. Polyploidization is genetically the most recognizable mechanism of speciation. A recent estimate suggests that 2–4% of speciation events in flowering plants involved polyploidization, but this is likely an underestimate because repeated origins of the same polyploid are not counted, and many polyploids in nature are not given distinct species status by taxonomists. Selection plays an important role in generating ecological differences between the new polyploid species and its ancestors, facilitating persistence.

Hybridization can also produce new species without polyploidy, by the production of novel hybrid genotypes whose traits may lie well beyond those of the parent species. The process is facilitated by the availability of a novel habitat to which hybrid genotypes are better suited than the parents. Spatial separation combined with strong selection on the new hybrid population reduces gene flow from the ancestral populations and can bring about further reproductive isolation as a by-product.

Sunflowers in the genus *Helianthus* provide the best known examples of hybrid speciation without polyploidization in nature driven in addition by strong selection. *H. annuus* and *H. petiolaris* have produced three identifiably distinct hybrid species, each confined to a unique environment stressful to both parental species (Figure 3). Experiments using artificially created hybrids have shown that some hybrid genotypes have much higher fitness than parental types when placed in these stressful environments.

Where to next?

Our understanding of the process of speciation has increased greatly since Darwin first proposed a central role for natural selection. Much of what we now know has come from research conducted over the past two decades. The picture emerging is that speciation is a process that results from the same forces responsible for most change within species: natural and sexual selection. Nonetheless, there are still many areas that require investigation.

The 'top down' or phenotypic approach to studying speciation has found evidence for selection on ordinary phenotypic characters shown also to underlie premating and postmating isolation. This approach has yielded little, however, about the genetic basis of reproductive isolation. For example, we do not know yet if species differences are based on many genes of small phenotypic effect, or if few genes of large effect are most important in causing divergence and reproductive isolation. This has made it difficult to pinpoint exactly how natural selection has led to divergence in most cases. Recent studies of speciation in

monkeyflowers and other taxa are helping to overcome this gap.

The 'bottom up' or genetic approach to studying speciation has hunted down genes responsible for premating and postmating isolation, and then shown that the gene sequences exhibit signatures of recent selection. But this approach has told us little about the nature of that selection. Is selection divergent or has divergence occurred under uniform selection? Was selection in response to environmental differences? Was it natural or sexual selection?

Finally, we still know little about how mate preferences evolve within and between populations during the process of speciation. Sexual selection by mate choice might be a near-universal process in speciation, but what drives the divergence of mate preferences to begin with?

Speciation study is in the midst of a surge of research effort, and part of the reason is that answers to many of these questions appear at last to be within reach. We expect that a combination of phenotypic and genetic approaches will soon close the gap between the genes and the mechanisms of selection, and yield a fuller account of how most species in nature have formed.

Further reading

Coyne, J.A., and Orr, H.A. (2004). Speciation, (Sunderland, MA: Sinauer).

Orr, H.A., Masly, J.P., and Presgraves, D.C. (2004). Speciation genes. Curr. Opin. Genet. Dev. *14*, 675–679.

Panhuis, T.M., Butlin, R., Zuk, M., and Tregenza, T. (2001). Sexual selection and speciation. Trends Ecol. Evol. *16*, 364–371.

Rieseberg, L.H. (1997). Hybrid origins of plant species. Annu. Rev. Ecol. Syst. *28*, 359–389.

Schluter, D. (2000). The Ecology of Adaptive Radiation, (Oxford: Oxford University Press).

Servedio, M.R., and Noor, M.A.F. (2003). The role of reinforcement in speciation: theory and data. Annu. Rev. Ecol. Evol. Syst. *34*, 339–364.

Turelli, M., Barton, N.H., and Coyne, J.A. (2001). Theory and speciation. Trends Ecol. Evol. *16*, 330–343.

Zoology Department and Biodiversity Research Centre, University of British Columbia, Vancouver, British Columbia, Canada, V6T 1Z4.
E-mail: albert@zoology.ubc.ca, schluter@zoology.ubc.ca

Correspondence

Shortest recorded vertebrate lifespan found in a coral reef fish

Martial Depczynski and David R. Bellwood

Extreme short lifespans are of interest because they mark current evolutionary boundaries and biological limits within which life's essential tasks must be successfully accomplished. Here we document the remarkable eight week lifespan of the coral reef pygmy goby *Eviota sigillata* [1] (Figure 1A): the shortest recorded lifespan for any vertebrate. Coral reef pygmy gobies spend their first three weeks as larvae in the open ocean before undergoing metamorphosis and returning to settle on the reef, where they mature within 1–2 weeks and have a maximum adult lifespan of just three and a half weeks.

The rapid transition from larvae to settlement and then maturity is recorded in the calcareous 'earstones' (otoliths) of fishes by the deposition of periodic concentric rings; these provide not only a sensitive record of time but a chronological 'black box' which imprints the age at which important events take place [2]. Each day, pygmy gobies lay down a new ring in their otoliths, much as a tree does for each year. We collected 319 *E. sigillata* specimens from the Great Barrier Reef over both summer and winter periods. Age was determined after validation of daily otolith ring deposition, and sexual maturity status identified using histological techniques. A settlement 'check' mark in the otolith at 23–27 days marked the transition from open ocean larvae to settlement on the reef. Analyses of the largest individuals showed that *E. sigillata* has a maximum total lifespan of 59 days, with a maximum post-settlement lifespan on the reef of just 35 days, of which at least 10 are taken to reach sexual maturity (Figure 1B). This provides the species with a remarkable three-week window in which to reproduce and contribute to the next generation.

Already constrained by time, the lifetime fecundity of *E. sigillata* is further restricted by small adult body sizes of 11–20 mm, limiting the number of eggs a female can produce. Yet pygmy gobies are an incredibly successful group, numbering some 70 species with a geographic distribution encompassing reefs across the Indian and Pacific Oceans [1]. To investigate lifetime fecundity, we bred pygmy gobies in captivity. Females were able to spawn successive clutches of 108–163 eggs (egg size 0.75 x 0.55 mm) within an 11–17 day period, suggesting that females have the potential to produce just three clutches in a lifetime — a little over 400 eggs. Given that larval mortality in reef fishes typically exceeds 95% [3], high larval survivorship is critical for this species, and males fan and guard their eggs until hatching, a reproductive strategy that greatly enhances offspring survivorship [4]. With an average generation time of just 49 days, *E. sigillata* may produce up to 7.4 generations per year.

For small species living in ecological settings where high extrinsic mortality rates exist, evolution often favors a 'live fast, die young' stratagem where rapid growth and maturation are favored [5,6], presumably as compensation for reduced life expectancy. For example, the vertebrate that previously had the shortest recorded lifespan and for which ageing data are available — the Turquoise killifish, *Notobranchius furzeri* — inhabits seasonal rain pools in equatorial Africa and must complete its reproductive cycle before these pools disappear [7]. Under laboratory conditions *N. furzeri* survives for only 12 weeks. Interestingly, recorded extremes in vertebrate life history traits, such as the smallest [8] and earliest maturing [9] vertebrates, are also found in coral reef fish species. Despite the prevalence of such life